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(57) Abstract

This invention refers to a method for Magnetic Resonance Imaging (MRI) using proton signals of paramagnetic metal-ion complexes, based on the use of Chemical Shift Imaging (CSI) techniques, as well as Magnetic Resonance Spectroscopy (MRS) for the control and recording of biological parameters.

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PARAMAGNETIC DIAGNOSTIC FORMULATIONS AND THEIR METHOD OF USE

This invention refers to a method for Magnetic Resonance Imaging (MRI) using proton signals of paramagnetic metal-ion complexes, based on the use of Chemical Shift Imaging (CSI) techniques, as well as Magnetic Resonance Spectroscopy (MRS) for the control and recording of biological parameters.

The use in medicine of a high number of these complexes is widely reported: for instance as stabilizers for the pharmaceutical preparations or antidotes in case of ingestion of toxic metal species.

Physiologically tolerable complexes formed by chelating agents and bi- or trivalent metal ions are used as diagnostic agents in imaging techniques such as X-ray, nuclear magnetic resonance (NMR) and scintigraphy.

In particular, magnetic resonance imaging (MRI) is a renowned powerful diagnostic procedure used in medical practice (see Stark, D.D., Bradley, W. G., Jr., Eds. "Magnetic Resonance Imaging" The C. V. Mosby Company, St. Louis, Missouri (USA), 1988) which relies on the use of paramagnetic pharmaceutical compositions, preferably containing chelated complexes of bi- or trivalent paramagnetic metal ions, usually belonging to the class of transition metals, or rare earth, with aminopolycarboxylic acids and/or their derivatives or analogues.

The images (basically coming from the NMR signal of water protons) are the result of a complex

interaction of different parameters, such as proton density and T_1 and T_2 relaxation times. A contrast enhancement can be obtained through the administration of exogenous chemical substances which significantly change the resonance properties of nearby water protons (see Lauffer, R.B. Chem. Rev. 1987,87,901).

Paramagnetic contrast media used in MRI modify the relaxation times of water protons in tissues, in which said contrast medium concentrates, and therefore can enhance the differences among different tissues, or between healthy and pathological tissues.

Due to the high capacity of gadolinium complexes of reducing the relaxation times of hydrogen nuclei of nearby water molecules through dipolar interaction, scientists have investigated, patented and published some works on these complexes (see Lauffer). And some of them have been approved as MRI contrast media (Gd-DTPA-DImeg, N-methylglucamine salt of gadolinium diethylenetriaminepentaacetic acid, MAGNEVIST®, Schering; Gd-DOTA-DImeg, N-methylglucamine salt of gadolinium 1,4,7,10-tetraazacyclododecan-1,4,7,10-tetracetic acid, DOTAREM®, Guerbet).

A list of significant patent documents showing the state of the art in this diagnostic field, even though uncompleted, is represented by: EP 71564 (Schering), US 4639365 (Sherry), US-A-4615879 (Runge), DE-A-3401052 (Schering) , EP 130934 (Schering), EP 65728 (Nycomed), EP 230893 (Bracco), US-A-4826673 (Mallinckrodt), US-A-4639365 (Sherry), EP 299795 (Nycomed), EP 258616 (Salutar), WO 8905802 (Bracco).

The choice of the suitable compound is based on

the evaluation of different parameters such as relaxivity, toxicity, distribution in the human body, excretion and so on. Three important properties are needed to use a complex, i.e. $Gd^{(3+)}$, as a potential MRI contrast agent. Firstly, a high thermodynamic stability (and possibly kinetic), that's to say a low tendency to release free $Gd^{(3+)}$ ion, highly toxic in vivo. Secondly, the presence of at least one water molecule directly coordinated to a metal in the inner coordination sphere and able to rapidly exchange with the bulk one. Thirdly, a high water solubility ($\geq$0.5 M).

The paramagnetic complexes, which up to now have been preferably used as contrast agents in MRI medical diagnosis, are chelated complexes of aminopolycarboxylic acids and/or their derivatives or analogues, with $Mn^{(2+)}$, $Fe^{(3+)}$ and $Gd^{(3+)}$ ions, which are the most widely investigated since they better meet the above mentioned needs. The other paramagnetic metal ions are not so efficient as relaxation agents, due to their very short relaxation times. On the contrary, a characteristic NMR property of complexes of these ions is the very high chemical shift of the magnetically active nuclei in the ligand. This property is partly transferred to the resonance characteristic of chemical species which interact with these complexes. When used for this aim in high-resolution spectroscopy, these complexes are called shift reagents (SR). Introduced during the '70s, basically as $Eu^{(3+)}$ and $Yb^{(3+)}$ complexes, they played a relevant role in the definition of organic molecule structures in solution

since they produce a shift of nearby nuclei resonance values, and therefore a consequent easier reading of spectra data.

Fluorinated compounds can represent an alternative approach to the diagnostic imaging through magnetic resonance in comparison to the a.m. $Gd^{(3+)}$,$Mn^{(2+)}$ and $Fe^{(3+)}$ paramagnetic compounds.

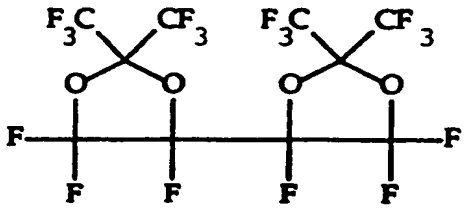
From the theoretical point of view, fluorinated substances used for the diagnostic imaging have some advantages: a) the absence, in fluorine spectral range, of signals coming from endogenous substances (fluorine is practically absent in biological systems); b) natural abundance (100%) and nuclear spin of ^{19}F (I=1/2). But these advantages are heavily limited by an important factor: the quite long relaxation times of fluorine nucleus, which causes a severe reduction of signal/noise ratio (S/N) of the obtainable image and/or a remarkable delay of analysis times.

Thanks to its good biocompatibility properties, PFOB (perfluorooctylbromide, currently under investigation as potential blood substitute) is the most investigated product. This substance, however, has some other drawbacks due to:

a) multiplicity of ^{19}F resonances generating a high signal dispersion;

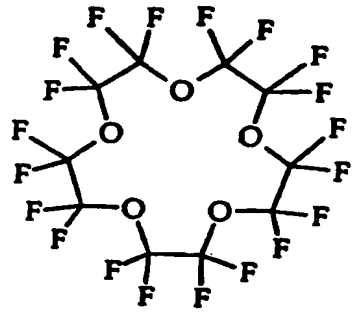
b) excretion difficulties.

Other compounds which try to overcome the above mentioned drawbacks have been prepared. For instance, PFBD (perfluoro-2,2,2',2'-tetramethyl-4,4'-bis(1,3-dioxolane) ($C_{10}F_{18}O_4$) has been prepared, having the following formula:



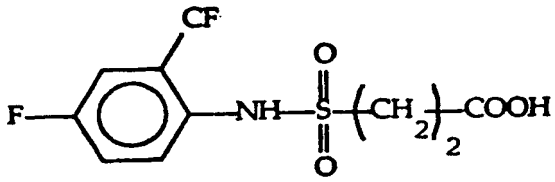
[Magn. Reson. Med., 20, 188 (1993)]

Another example comes from perfluoro-15-crown-5, constituted by 20 F,



claimed as MRI contrast agent in patent EP 307863.

Some of these fluorinated compounds have been proposed as diagnostic probes in MR spectroscopy (MRS). In short, they are compounds containing at least two different kinds of fluorine atoms, whose different resonancesl can be used as chemical sensors of biological parameters, such as pH, pO_2, pCO_2, T, or more generally of the chemico-physical environment of organs or tissues. For instance patent applications EP-A-447013 and AU 633850 claim fluorinated compounds of the following type:



[Book of Abstracts, XIth Meeting SMRM, p.3413]

to be used as sensors for measuring the pH in living tissues.

We have now unexpectedly found out, and this is one of the most remarkable aspect of this invention, that this type of application can be also performed in proton NMR spectroscopy, by using paramagnetic complexes endowed with shift reagent properties.

In addition, and this is another aspect of this invention, if in the paramagnetic metal complex, there are protons characterized by sufficiently intense signals and chemical shifts outside the interval of proton signal of nearby water and of the biological system constituents, the signal difference between bulk water and said paramagnetic complex protons can be exploited to obtain, thanks to Chemical Shift Imaging (CSI) technique, excellent images for the distribution of the contrast medium in the tissue or the organ under exam. Therefore, in contradiction to the universally accepted teaching of the prior art, said images comes from the proton resonance of the chelating or complexing agent and not from those belonging to water of the system under exam.

As far as MRS is concerned, these complexes are used as probes to measure biological parameters such as for instance pH, pO_2, pCO_2, T, in the tissues where the

contrast medium preferentially concentrates. These applications, even if similar to those proposed for fluorinated derivatives, do not present the drawbacks belonging to these last ones, thus representing an unexpected and a remarkable technical improvement to the state of the art.

A remarkable advantage given by the use of the above mentioned compounds in Chemical Shift Imaging, resides in the extremely short T_1 values of paramagnetic compounds resonances which allow very rapid pulsations. This allows a high S/N ratio of the obtainable image, and the possibility of operating at low concentrations of contrast agent, with acceptable analysis times.

As a matter of example, if we use a complex of a lanthanum ion with the usual administered dosis in MRI, 0.1 mmol/kg, and we hypothesize an entirely intravascular distribution, we can reach a concentration of 1.5 mM, which generates a spectrum showing a good S/N ratio in few minutes (2-6), obtaining images showing a good distribution of the contrast agent.

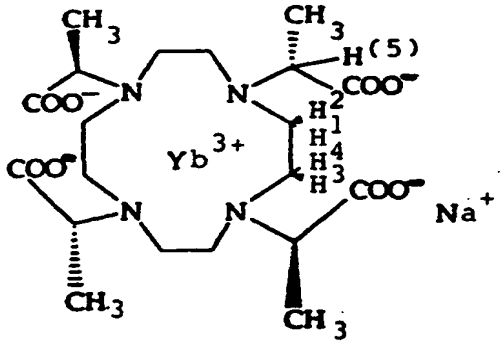
As far as the application of such complexes in MRS are concerned, they can be properly used as probes to measure the temperature of the districts under exam. As a matter of fact it is known that the paramagnetic shift heavily depends from temperature, through the contact factor (Curie's behaviour, $1/T^2$ dependent), and through the dipolar factor ($1/T$ dependent).

Through suitable functionalizations, the paramagnetic shift can be made dependent from pH or

from specific interactions with the substrates presenting the organs or tissues under exam.

This invention refers to a method of use of proton signals of paramagnetic metal-ion complexes in MRI, thanks to the use of Chemical Shift Imaging techniques, as well as in MRS for the control and recording of biological parameters.

A particularly suitable example which highlights the originality of this invention and its high potential diagnostic application is the Yb (III) complex of [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',-α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (DOTMA) as hereunder represented. This product is cited in one article only (Brittain, H. G.; Desreux, J. F., Inorg. Chem., **1984**, 23, 4459), without mentioning its preparation and chemico-physical characteristics, apart from ^{1}H-RMN spectrum and a study concerning conformational isomers in solution.



As far as the ligand type is concerned, that's to say DOTMA, it must be cited one preparation method

published on Inorg. Chem., 32, 2912, 1993, authors Tweedle M. F. et al., starting from [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid, tetrabenzyl ester (DOTMA-TBE).

The presence of 12 equivalent protons belonging to the four $-CH_3$ groups, originates an intense unitary signal at about -14.2 ppm under the described conditions of Fig. 1, Example 3, providing a good S/N ratio in MRI exams.

In this case, the metal outdistances the signal of the four methyls of the propionic chain from water protons by about 20 ppm, so that the hydrogen nuclei of these methyls are made "different" from the hydrogen nuclei of bulk water, making this difference exploitable with CSI.

The same complex can be used in MRS techniques as probe for determining for instance the temperature of the district where the complex accumulates. For instance the chemical shift difference among non-equivalent protons such as H(1) and H(5) is dependent from the temperature of biological districts under exam. Therefore very interesting and useful information can be gathered by comparing the different proton resonances, thanks to the use of this technique.

Suitable paramagnetic complexes of this invention are substantially constituted by: a) a paramagnetic metal ion with atomic number selected between 21 and 29, 42, 44 and between 58 and 83 except for 64 and 71; b) a chelating agent in which one or more hydrogen atoms originate, after chelation, NMR signals having

chemical shifts different from those of nearby water and being outside the range of the proton signals of the constituents of biological systems (said interval is usually between 0 and 10 ppm).

Of course, these paramagnetic complexes can be neutralized with organic and/or inorganic physiologically compatible bases or acids. Particularly preferred metal ion are V^{3+}, Mn^{3+}, Fe^{2+}, Fe^{3+}, Co^{+}, Co^{2+}, Ni^{2+}, Mo^{3+}, Ru^{3+}, W^{3+}, Re^{3+}, Os^{3+}, Yb^{3+}, Dy^{3+}.

Preferred ligands are chelating agents containing N, O, P and S as donor atoms, both linear and cyclic. For instance they can comprise catecholates, salicylates, di- and polyamines, diphosphines, Schiff bases, amino acids, ß-diketonates, ß-thioketonates, ß-thiocarbamates, polyaminocarboxylates, -phosphates, -phosphinates, and so on.

Useful compounds for these applications are also water-soluble porphyrins (and their analogues). Also water-soluble organometallic compounds meet the above mentioned aim.

In addition complexes with a balance between low and high spin shapes must be included. Due to the large differences in the NMR parameters of these two shapes, these systems can experience a wide chemical shift variation of their resonances according to the different environmental conditions. Suitable substituents can be introduced on these complexes to enable the transfer of environmental parameter variations (T, pO_2, pH,....) to the concentration variation of balanced shapes. Absolutely non-limiting examples of complexes characterized by this type of

spin balance can be: a) bis-[tris(1-pyrazoyl)borate]Fe(II); b) tris(N,N-dithiocarbamate) Fe(III).

EXAMPLE 1

Yb^{3+} complex with [1R-(1R*,4R*,7R*,10R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid sodium salt.

A) [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,-10-tetraacetic acid.

A solution of 43.2 g of (S)-2-chloropropionic acid (prepared according to Fu, S. C., J. Am. Chem. Soc., **1954**, 76, 6054) (0.40 mol) in 60 ml of water, is cooled to 5°C, and then the pH is adjusted to 5 with 20% NaOH without exceeding 5°C. 13.8 g of 1,4,7,10-tetraazacyclododecane (prepared according to Atkins T. J. et al., Org. Synth., **1978**, 58, 86) are added in portions and the solution is heated to 70°C for 24h, by keeping the pH at 10 by addition of 20% NaOH. After filtration of the solid, the solution is concentrated and to the residue are added further 10 g of (S)-2-chloropropionic acid (0.09 mol). Then it is retaken to the previous conditions (pH 10, 70°C) for additional 15 h, obtaining another portion of precipitate which is filtered. The two resulting precipitates are collected and dissolved in 1 l of water and the solution is acidified at pH 4 (through 37% HCl) and then electrodialyzed. The resulting solution is concentrated and the residue is diluted with cold EtOH. After filtration, 10.2 g (0.022 mol) of the desired compound are obtained.

Yield: 28% m.p.: > 250°C

K.F.: 0.30% (w/w)

HPLC: 98.2% (% area)

0.1N NaOH: 99.0% (w/w)

Elemental Analysis	C	H	N
% calc.:	52.16	7.88	12.17
% found:	51.91	7.90	12.12

^{1}H-RMN, ^{13}C-RMN, IR and MS spectra are consistent with the indicated structure.

B) Yb^{3+} complex of [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraaza-cyclododecan-1,4,7,10-tetraacetic acid sodium salt

2.3 g of [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (0.005 mol) are suspended in 20 ml of water and solubilized at pH 6.5 by addition of 6 ml of 2N NaOH (0.012 mol). To the resulting solution, 1.94 g of $YbCl_3 \cdot 6H_2O$ (marketed product) (0.005 mol) are added in 10 ml of water by checking that the pH solution drops down to about 4. 2N NaOH is dropwise added to the mixture up to pH 5. The solution is kept at a constant pH (pH 6.5) by addition of 4ml of 2N NaOH (0.008 mol) during 10 h. After filtration and electrodialysis, the solution is taken to pH 6.5 through 1N NaOH and concentrated to dryness. 1.65 g (0.00253 mol) of the desired compound are obtained.

Yield: 51% m.p.: >250°C

K.F.: 2.89% (w/w)

Elemental Analysis	C	H	N	Na	Yb	Cl
% calc.:	36.81	4.94	8.59	3.52	26.52	
% found:	36.05	5.15	8.49	3.02	25.18	<0.10

^{1}H-RMN, ^{13}C-RMN, IR and MS spectra are consistent with the indicated structure.

EXAMPLE 2

Europium complex with [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid sodium salt.

In accordance with the procedure described in EXAMPLE 1, 2.3 g of [αR*(αR*, α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (0.005 mol) in 20 ml of water are reacted with 1.83 g of $EuCl_3 \cdot 6H_2O$ (marketed product) (0.005 mol) in 10 ml of water. 1.50 g of the desired compound are obtained (0.00237 mol).

Yield: 47% m.p.: >250°C (dec.)

K.F.: 3.06% (w/w)

Elemental Analysis	C	H	Eu	N	Na	Cl
% calc.:	38.04	5.11	24.06	8.87	3.64	
% found:	37.00	5.69	23.26	8.63	3.04	<0.10

^{1}H-RMN, ^{13}C-RMN, IR and MS spectra are consistent with the indicated structure.

EXAMPLE 3

Proton spectra recorded by AMS-400 spectrometer, ^{1}H 400,13 MHz; ^{13}C 100.61 MHz; internal reference: H_2O 4.8 ppm.

Figure 1: proton spectrum of a 20mM solution of serum Yb-DOTMA SERONORM-HUMAN™ (NYCOMED).

Figure 2: proton spectrum of a 0.12 M solution of Yb-DOTMA in 0.5 ml of D_2O.

CLAIMS

1. A method for obtaining images of organs, tissues, biological districts of human or animal body through nuclear magnetic resonance (NMR), comprising,

a) administration of a physiologically tolerable pharmaceutical formulation, containing at least a paramagnetic chelating agent/complex, or a salt thereof, wherein:

1) the paramagnetic part is constituted by a paramagnetic metal ion selected from thos having atomic number between 21 and 29, 42, 44 and between 58 and 83 except for 64 and 71,

2) the ligand, or the chelating or the complexing part is constituted by an organic, linear or cyclic molecule, in which the electrodonor nuclei are O, N, S, P,

being such paramagnetic chelate/complex characterized by one or more proton resonance signals falling outside the interval occupied by the signals of the nearby water protons and of the signals of the protons of the biological systems,

b) obtention of said images by "Chemical Shift Imaging" techniques.

2. Pharmaceutical formulations according to claim 1 wherein the paramagnetic metal ion is selected from V^{3+}, Mn^{3+}, Fe^{2+}, Fe^{3+}, Co^{+}, Co^{2+}, Ni^{2+}, Mo^{3+}, Ru^{3+}, W^{3+}, Re^{3+}, Os^{3+}, Yb^{3+}, Dy^{3+}.

3. Pharmaceutical formulations according to claim 1 wherein the chelating/ligand part is selected from: aminopolycarboxylic acids and their derivatives,

catecholates, salicilates, di- and polyamines, diphosphines, Schiff bases, aminoacids, ß-diketonates, ß-thioketonates, ß-thiocarbamates, polyaminocarboxylates, -phosphates, -phosphinates, porphyrins and water-soluble organometalic compounds.

4. Pharmaceutical formulations according to claim 3 wherein the chelating agent is selected from: [αR*(αR*,α'R*,α''R*,α'''R*)]-(α,α',α'',α''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid, bis-[tris(1-pyrazoil)boric acid, tris-N,N-dithiocarbamic acid, and their derivatives.

5. Pharmaceutical formulations according to claim 1 wherein the paramagnetic chelate/complex can be salified with physiologically tolerable organic and/or inorganic bases or acids.

6. Use of pharmaceutical formulations according to claim 1 to obtain images of organs, tissues, biological districts of human or animal body by using "Chemical Shift imaging" techniques.

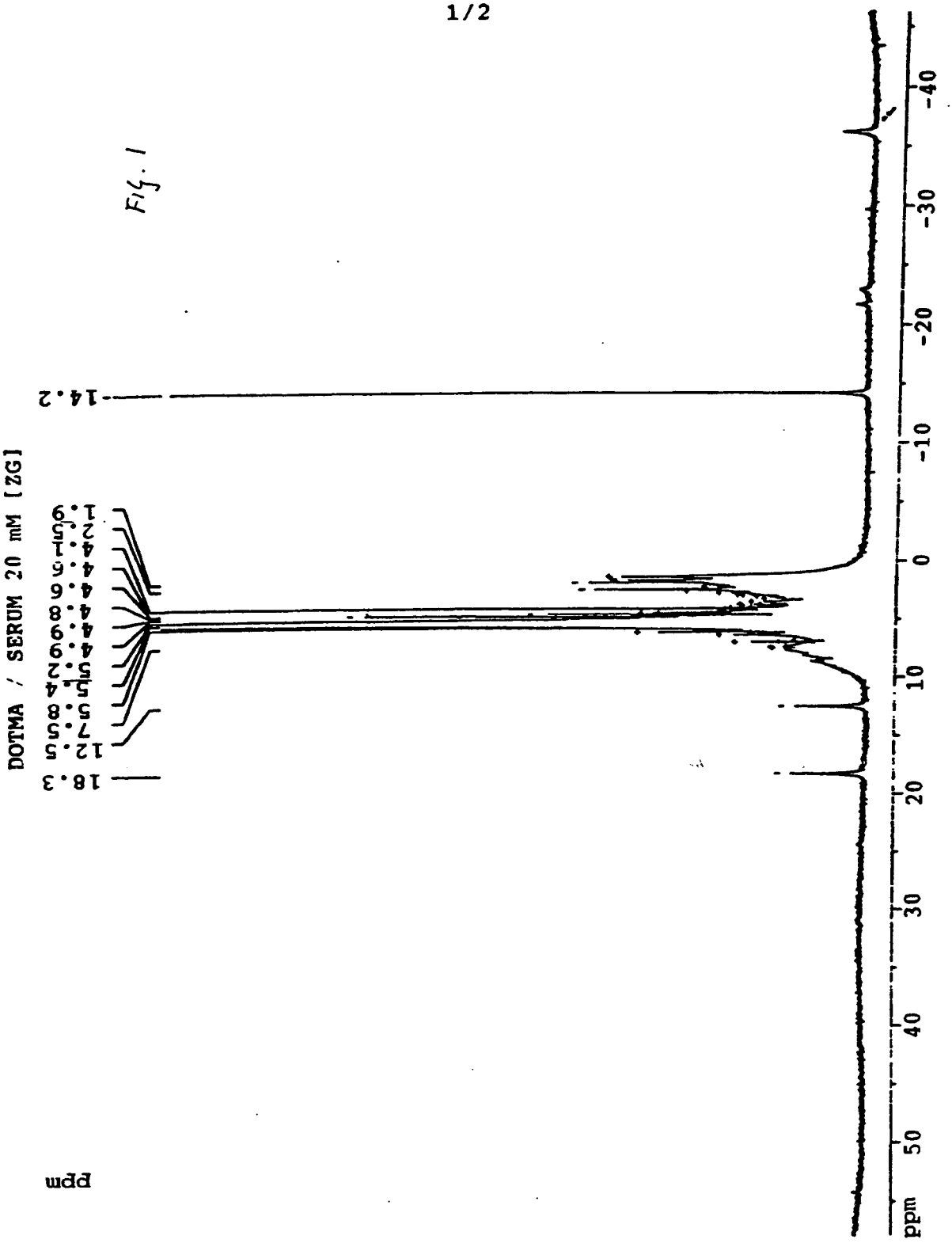
7. Use of paramagnetic chelate/complexes according to claim 1 and/or their physiologically tolerable salts for the preparation of pharmaceutical formulations to be used in "Chemical Shift imaging" techniques.

8. A method for obtaining information on biological parameters of living tissues through Magnetic Resonance Spectroscopy (MRS), comprising the preliminary administration of pharmaceutical formulations according to claim 1.

9. A method for measuring the pH in living tissues through MRS, comprising the preliminary administration of pharmaceutical formulations according to claim 1.

10. A method for measuring the temperature in living tissues through MRS, comprising the preliminary administration of pharmaceutical formulations according to claim 1.

Fig. 1



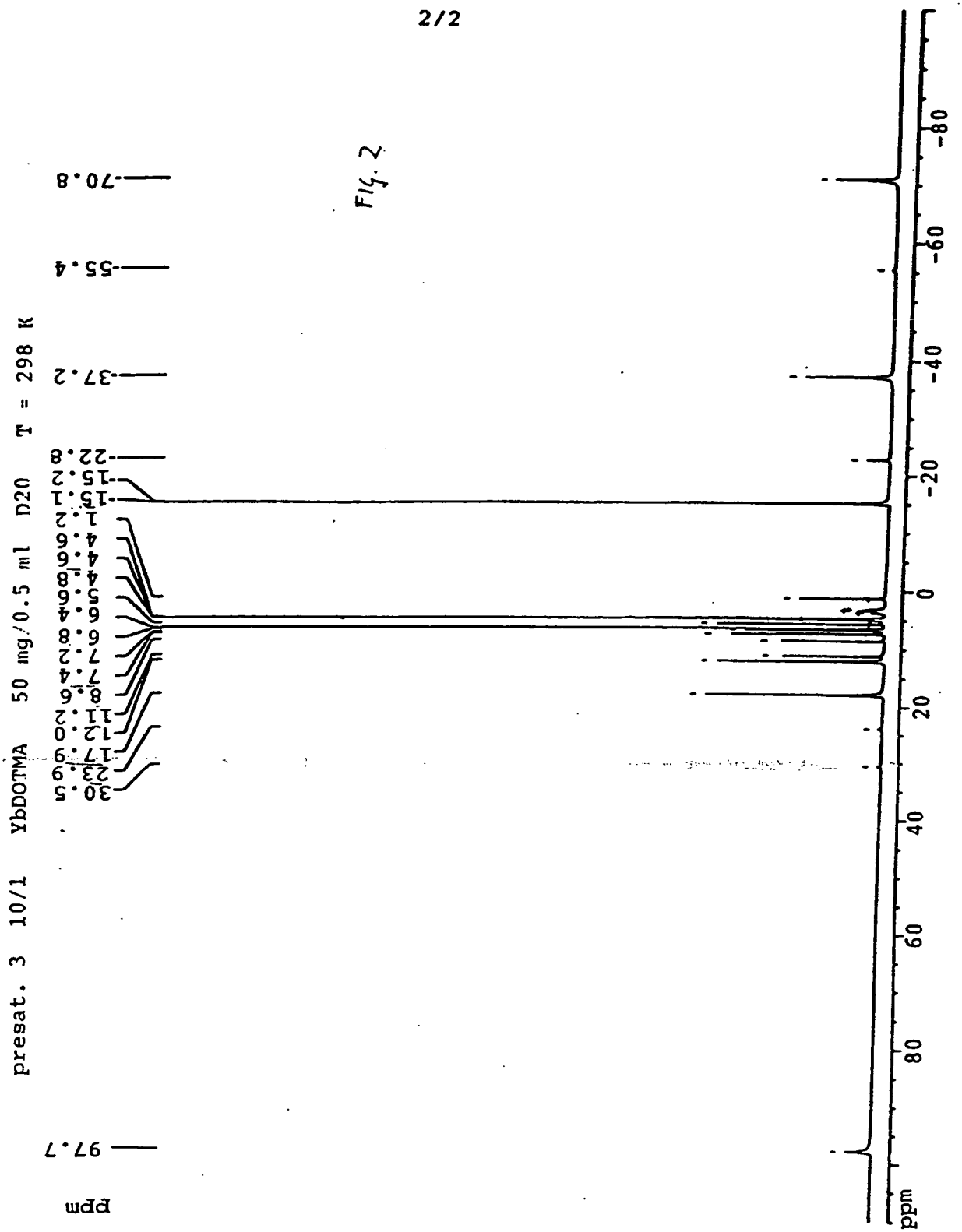


Fig. 2

Inte. ional Application No
PCT/EP 94/03944

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP-A-0 122 000 (CHILDRENS HOSP MEDICAL CENTER) 17 October 1984 see page 12, line 13 - line 20 see page 39, line 1 - line 10; claims	1,2,5-10
A	DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US AN=118:55405, INGWALL, JOANNE S. 'Measuring cation movements across the cell wall using NMR spectroscopy: sodium movements in striated muscle' see abstract & NMR (1992), 28(IN-VIVO MAGN. RESON. SPECTROSC. III), 131-60 CODEN: NBPPD3;ISSN: 0170-5989, 1992	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO-A-94 27977 (SCHERING AG ;PLATZEK JOHANNES (DE); RADUECHEL BERND (DE); NIEDBALL) 8 December 1994 see claims	1-8,10
E	WO-A-94 27978 (SCHERING AG ;PLATZEK JOHANNES (DE); RADUECHEL BERND (DE); NIEDBALL) 8 December 1994 see claims	1-8,10
X	EP-A-0 095 124 (BRUKER MEDIZINTECH) 30 November 1983 see page 11, paragraph 2 see page 12, paragraph 1; claims 6,8	1-3,5-10
X	DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US AN=118:92999, HUTCHISON, ROBERT B. ET AL 'Suppression of sodium nuclear magnetic resonance double-quantum coherence by chemical shift and relaxation reagents' see abstract & J. CHEM. PHYS. (1992), 97(12), 8934-40 CODEN: JCPSA6;ISSN: 0021-9606, 1992	1-3
X	DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US AN=116:169116, KOHLER, SUSAN J. ET AL 'In vivo sodium chemical shift imaging' see abstract & MAGN. RESON. MED. (1992), 23(1), 77-88 CODEN: MRMEEN;ISSN: 0740-3194, 1992	1-3,5-9
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Inte onal Application No
PCT/EP 94/03944

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US AN=92008702, see abstract & EUR. J. VASC. SURG., vol. 5, no. 4, August 1991 pages 383-396, MOHIADDIN R.H. 'm.r. orphological chemical shift imaging in peripheral vascular disease.'	1
X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US an=92063380, see abstract & BR. J. RADIOL., vol. 64, no. 766, October 1991 pages 923-928, SMITH S. R. ET AL. 'TUMOUR PH AND RESPONSE TO CHEMOTHERAPY: AN IN VIVO 31P MR SPECTROSCOPY STUDY IN NON-HODGKIN'S LYMPHOMA.'	1
X	US-A-4 915 933 (MATWIYOFF NICHOLAS A) 10 April 1990 see column 4, line 55 - column 5, line 31; claims; tables	1-10
X	EP,A,0 135 125 (DU PONT) 27 March 1985 see page 4, line 1 - page 7, line 27; claims	1-10

INTERNATIONAL SEARCH REPORT

I national application No.
PCT/EP 94/03944

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1,8-10
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1,8-10 are directed to a method of treatment of (di
agnostic method practised on) the human/animal body the search has been car
ried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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